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MORPHINE INDUCES AN INTRACELLULAR ALKALINIZATION IN
BOVINE AORTIC ENDOTHELIAL CELLS (BAECs)

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The resting intracellular pH (pHi) of BAECs, by using BCECF fluorescence, at 37 °C in Na⁺ Hanks' was 7.22±0.03. Cells which had been acid-loaded recovered from the intracellular acidification in Na⁺ Hanks' in a [Na⁺]_o-dependent and amiloride-sensitive manner. Recovery from acidification had an apparent Km for Na⁺ of 40 ± 10 mM and Ki for amiloride of 26±4 μM. Morphine (50 μM, 20 min, 37 °C) increased the pHi to 7.55 ± 0.05. Naloxone (50 μM) given 5 min before morphine (50 μM) blocked this effect, indicating that this was an opiate receptor-mediated phenomenon. To determine if morphine activated the Na⁺/H⁺ exchanger, pHi was monitored in Na⁺-free Hanks', acidic Na⁺ Hanks' or amiloride-containing Na⁺ Hanks'. The alkalization produced by morphine was not observed under all these circumstances. These data suggest morphine activates the Na⁺/H⁺ exchanger via opiate receptors.

INTRODUCTION

A monolayer of vascular endothelial cells lines the inner surface of the blood vessels and has broad metabolic functions. The intracellular pH (pHi) must be maintained in a narrow range to provide an environment for various intracellular reactions (Moolenaar, 1986). In many cells, pHi homeostasis is maintained by 3 defined ion transporters: the Na⁺/H⁺ exchanger, Na⁺-dependent and Na⁺-independent Cl⁻/HCO₃⁻ exchangers (Grinstein et al., 1989). The presence of the Na⁺/H⁺ exchanger has been demonstrated in rat brain capillary endothelial cells *in vivo* and nonadherent bovine aortic endothelial cells *in vitro*.

Previous studies showed that δ-opiate agonists elicited a cytoplasmic alkalization in NG108-15 cells which was concentration-dependent and reversed by naloxone (Isom et al., 1987). We report, herein, that morphine can alkalize adherent BAECs via activation of Na⁺/H⁺ exchanger.

MATERIALS AND METHODS

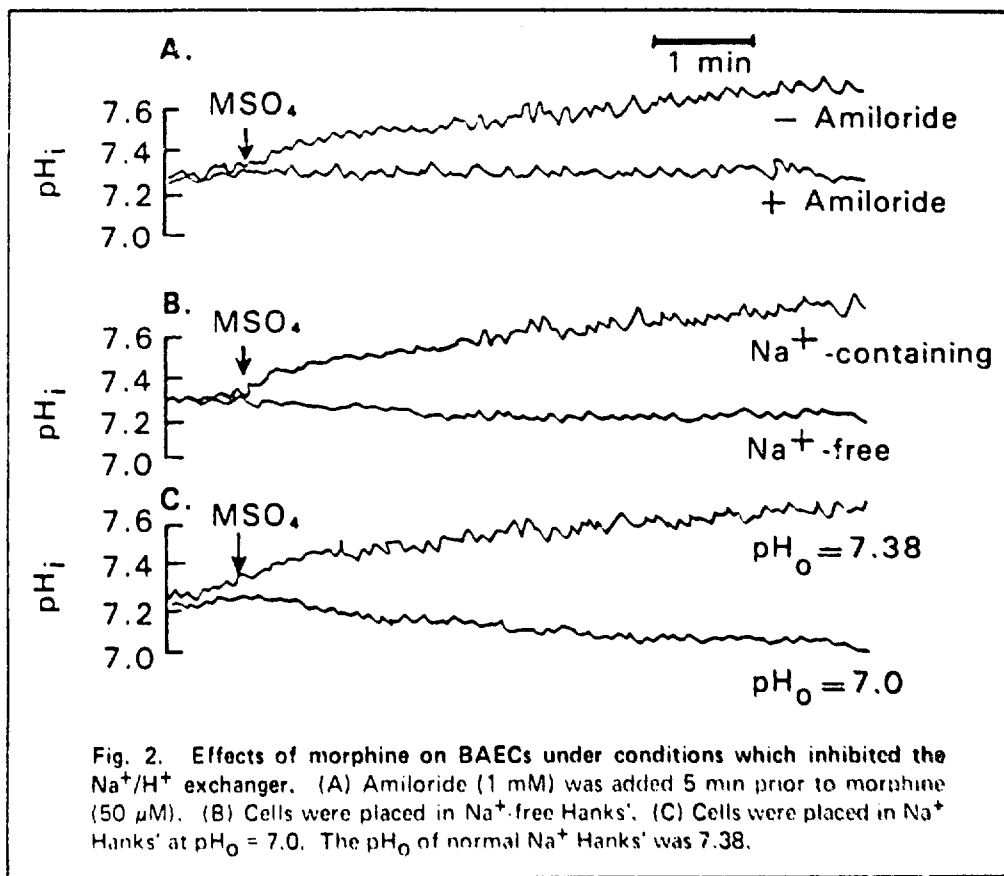
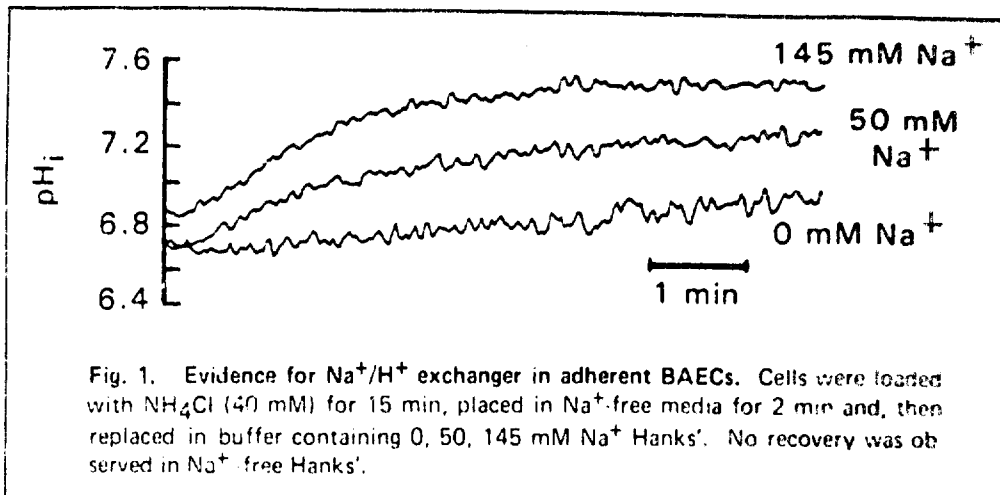
BAECs were obtained from bovine aorta by digestion with 0.2% collagenase (Eskin et al., 1978). The cells were cultured in Dulbecco's modified Eagle medium supplemented with 2 mM glutamine, 4.5 g/l glucose, 25 mM HEPES, 10% fetal bovine serum, penicillin (50 μ g/ml) and streptomycin (50 U/ml) (Gibco, Grand Island, NY). Typical cobblestone morphology was observed and angiotensin converting enzyme activity was measured. The cells at passage 6-10 were used for experiments. To measure pHi, confluent monolayers of BAECs were incubated with 5 μ M BCECF acetoxymethyl ester in Na⁺ Hanks' (in mM, 148 NaCl, 4.8 KCl, 1.2 MgCl₂, 1.8 CaCl₂, 20 HEPES, pH=7.38) at 37 °C for 15 min. The fluorescence signal was measured at 37 °C on a SLM 8000C spectrofluorometer as the ratio of emission at 529 ± 4 nm for excitation at 505 ± 4 and 442 ± 4 nm. The fluorescence signal was calibrated by using nigericin (3 μ M) and valinomycin (3 μ M). To determine the recovery rate from acidification in acid-loaded cells, cells were incubated in Hanks' with 40 mM NH₄Cl for 15 min. Then, cells were placed in Na⁺-free Hanks' (N-methyl-(+)glucamine substituted for Na⁺) for 2 min. Recovery from acidification was measured when cells were placed in Hanks' containing different concentrations of Na⁺. The initial rate of recovery was determined.

RESULTS

The resting pHi in adherent BAEC at 37 °C in Na⁺ Hanks' is 7.22 ± 0.03 (n=32). Amiloride (100 μ M), a blocker of Na⁺/H⁺ exchanger, added to the cells did not change the resting pHi indicating the Na⁺/H⁺ exchanger was inactive in resting cells. Acid-loaded cells had a pHi of 6.76 ± 0.05 (n=22) in Na⁺-free (NMG) Hanks' but recovered their pHi to 7.2 within 2 min after being placed in Na⁺ Hanks'. Cells which had been acid-loaded recovered from the intracellular acidification in Na⁺ Hanks' in a [Na⁺]_o-dependent manner (Fig. 1) and had an apparent Km for Na⁺ of 40 ± 10 mM. Amiloride (1 mM) inhibited the initial rate of recovery from acidification in Na⁺ Hanks' by 94%; inhibition was concentration-dependent with an apparent Ki for amiloride of 26 ± 4 μ M (data not shown). The results suggest the pHi recovery from an imposed acid-loading is mediated by the Na⁺/H⁺ exchanger in BAECs.

When morphine (50 μ M, 20 min) was added to the cells, an alkalization to 7.55 ± 0.05 pH units (n=10) was produced (Fig. 2A) and took an hour to return to its resting pHi. Naloxone (50 μ M) given 5 min before morphine (50 μ M) blocked this effect (naloxone: 7.32 ± 0.06 ; naloxone+morphine: 7.29 ± 0.05 , n=5 for both groups), suggesting that this was an opiate receptor-mediated phenomenon. It should be noted that naloxone itself increased pHi by 0.14 ± 0.05 units (n=5). To determine if morphine activated the Na⁺/H⁺ exchanger, pHi was monitored in the presence of amiloride by which Na⁺/H⁺ exchanger was blocked. Morphine

did not induce changes in pH_i (Fig. 2A). Experiments were also conducted in Na^+ -free Hanks' in which the activity of the Na^+/H^+ exchanger was inhibited by removing extracellular Na^+ . Morphine-induced



alkalinization was not observed (Fig. 2B). Similarly, an increase in extracellular proton which can block antiporter-dependent H^+ efflux also inhibited morphine-induced alkalinization (Fig. 2C). The lack of an effect of morphine on pHi after the blockade of Na^+/H^+ exchanger suggests that morphine alkalinized BAEC via an activation of Na^+/H^+ exchanger.

DISCUSSION

The resting pHi of adherent BAECs is 7.22 ± 0.03 which is in agreement with the resting pHi of nonadherent BAECs as shown by Kitazono et al. (1988). Morphine induced an intracellular alkalinization which took 1 hr to return to its resting pHi. The effect was via Na^+/H^+ exchanger since the presence of amiloride prevented the effect. The view was further supported by two other observations. That is, removal of extracellular Na^+ or an increase of extracellular proton inhibited morphine-induced alkalinization. This is not unique to morphine. A similar effect has been reported in many different cells treated with agents such as growth factors, mitogens, tumor-promoting agents (Grinstein and Rothstein, 1986). The underlying mechanism(s) of morphine-induced alkalinization is not understood. It is possible that morphine activates the Na^+/H^+ exchanger either by modifying the apparent affinity for Na^+ or H^+ , or by changing the maximal rate of exchange. Our preliminary data have also indicated that opiate receptors are on the cell membrane in BAECs (data not shown). Pretreatment of naloxone prevented the alkalinization suggesting that morphine-activated Na^+/H^+ exchange is coupled to opiate receptors. It will be interesting to study if the alkalinization produced by opiates in BAECs is involved in changing the blood pressure which occurs following administration of opiates *in vivo*.

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